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THE EFFECTS OF SOME HORMONES AND RELATED COMPOUNDS ON THE BACTERIA-FREE CULTURE OF *ENTAMOEBA INVADENS* AND FREE-LIVING AMOEBAE[1]

BY ELLIOTT LESSER

Abstract

Some 3-keto-Δ^4 steroids and diethylstilboestrol significantly inhibited the growth of *Entamoeba invadens* in a bacteria-free medium containing fresh liver. The inhibitory action of the steroids is not as great in the presence of fresh liver as in the presence of bacteria. The possible relationship between the degree of inhibition and steroid structural formulae is considered. No compound tested affected the growth of *Acanthamoeba castellanii* or *Hartmanella rhysoides*.

Introduction

In vivo experiments involving the vertebrate endocrine system and endoparasites have shown that at least some parasites are influenced either beneficially or adversely by their host's endocrine glands (1–7, 9–20, 22, 24–31, 34–37, 39, 41–44). In *in vivo* experiments, however, the administration of gland extracts or hormones to an animal or the removal of a particular endocrine gland, or both these procedures, has many varied effects, some (if not most) of which are unknown. Therefore, it is not certain whether the parasite under observation is affected directly by the hormone concerned or indirectly by an induced physiological change in the host. *In vitro* studies may, however, indicate whether the effect on the parasite is a direct one. Similarly, studies along this line may lead to a better understanding of host–parasite relationships and parasite physiology. Investigations involving *in vitro* experiments with endoparasites are practically limited to certain protozoa, and *Entamoeba invadens* was chosen for this study because it is one of the few species easily cultivated. Free-living amoeba were used for comparison with the parasitic species.

Thus far *in vitro* studies have shown that insulin in the presence of bacteria supports the growth of the human parasite, *Entamoeba histolytica* (32, 33), and that oestradiol, oestradiol-3-benzoate, and oestriol do not affect the bacteria-free *in vitro* growth of *Trichomonas vaginalis* (21). In a previous paper (23) it was shown that certain 3-keto-Δ^4 steroids inhibited the growth of

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E. invadens (an amoeba parasitic in certain reptiles) in the presence of *Escherichia coli, Serratia marcescens*) (= *Bacillus prodigiosus*), and a mixed bacterial flora.

This paper is concerned with a study of the inhibitory action of certain steroids and nonsteroid hormones on the bacteria-free *in vitro* growth of *E. invadens* and two species of free-living amoebae: *Acanthamoeba castellanii* and *Hartmanella rhysoides.*

Materials and Methods

(*a*) *Amoebae*

Two strains (A and B) of *E. invadens* were used in these experiments. Strain A was originally obtained in 1947 from the National Institutes of Health, Bethesda, Maryland, U.S.A. Strain B was isolated at the Institute of Parasitology in 1952 by Dr. M. J. Miller from a naturally infected water-snake from Florida.

The two species of free-living amoebae were supplied by Drs. S. H. Hutner and J. Storm of Haskins Laboratories, New York City.

(*b*) *Culture Media*

Strains A and B were each cultured in a bacteria-free medium containing fresh liver (23).

The free-living amoebae were cultured in a bacteria-free medium (the formula of which was supplied by Dr. J. Storm) consisting of 2.0% nutrient peptone (Difco proteose peptone No. 3 was used) and 0.1% skim milk powder. Sodium G penicillin, 4000 units/ml., and dihydrostreptomycin sulphate, 1000 units/ml. were added, but are not required to ensure sterility.

(*c*) *Steroids and Nonsteroid Hormones Tested*

Androstane-17-β-ol-3-one (Merck)
Androstene-3, 17-dione (Frosst, Ciba)
Androstene-3β, 17α-diol *cis* (Frosst)
Androstene-3β, 17β-diol *trans* (Frosst)
Cholestenone (Ayerst)
Cholesterol (Ayerst)
Compound A acetate (Merck, Ciba)
Compound F (Ciba)
Compound S (Ciba)
Cortisone acetate (Merck)
Dehydroepiandrosterone (Frosst)
Desoxycorticosterone (Frosst)
Desoxycorticosterone acetate (Frosst)
Diethylstilboestrol (Frosst)
Diethylstilboestrol sulphate (Frosst)
Dihydrotestosterone propionate (Ciba)
Oestrone (Ayerst)
Pregnane-17α, 21-diol-3, 11, 20-trione (Merck)
Δ^4-Pregnene-17α, 21-diol-3, 20-dione (Merck)
Δ^4-Pregnene-11β, 17α, 21-triol-3, 20-dione (Merck)
Progesterone (Ayerst)
Testosterone (Ayerst)
Thyroxine (Prof. C. P. Leblond, McGill University).

(d) Testing Procedure

All the compounds tested were in 1.0% saline suspension, except diethylstilboestrol sulphate which was in water solution. In the experiments with *E. invadens* all the compounds were used in 0.0005 gm. per ml. concentrations in the culture media, except thyroxine which was in a 0.0002 gm. per ml. concentration. In the experiments with the free-living amoebae the compounds were used in concentrations of 0.001 gm. per ml. in the culture media, except thyroxine which was used as above.

Each culture tube received a total of 10 ml. of medium including a sufficient quantity of the compound being tested in the concentration stated above. Control cultures received equivalent quantities of 1.0% saline.

Compounds not received in sterile condition were sterilized in powder form in a steam sterilizer (no pressure) for 60 min. The compounds were (prior to sterilization) placed in glass vials with rubber caps. A small hypodermic needle plugged with cotton was then inserted into each rubber cap. The needles and caps were in turn covered with brown paper tied with string. After sterilization the paper and needles were removed.

Because of limited available supplies of some compounds they could not be tested with all the amoebae.

The average number of *E. invadens* trophozoites per mm.^{3} in each culture tube was determined daily over a seven-day period by using a Spencer – bright line haemacytometer. The number of amoebae found in four haemacytometer readings were averaged and the number of amoebae per mm.^{3} calculated.

The free-living amoebae were not counted. Their development was observed over a seven-day period by examining the culture tube under the low power objective of the microscope. The amoebae adhere to the sides of the tube and can easily be seen.

Results

Table I illustrates the relative effects of the compounds tested on the growth of Strains A and B.

The compounds designated by an asterisk were tested with both species of free-living amoebae and exerted no observable effect on the growth of these amoebae.

Table II indicates the structural aspects of those steroids which, when compared to their respective controls, produced a highly significant inhibition ("P" < 0.001, according to the "t" test) of Strains A and B.

Table III indicates the structural aspects of steroids which did not produce a significant inhibition ("P" < 0.05) of Strains A and B. (There were no "P" values obtained between 0.001 and 0.05.)

TABLE I

INHIBITION OF *E. invadens* GROWTH *in vitro* BY STEROIDS AND NONSTEROID HORMONES

Compound	No. cultures tested:		Av. No. amoebae/mm.^{3}		Per cent inhibition	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Diethylstilboestrol*	7	4	0	0	100	100
Controls	8	4	8.36	5.64		
Compound A acetate (Kendall)	6	—	0.73	—	92.8	—
Controls	8	—	10.14	—		
Cortisone acetate*	13	7	0.71	0.30	92.3	96.4
Controls	12	9	9.23	8.70		
Desoxycorticosterone*	16	10	0.76	1.08	92.0	86.2
Controls	16	14	9.49	7.82		
Desoxycorticosterone acetate*	15	10	0.76	0.97	90.8	87.6
Controls	16	14	8.30	7.82		
Cholestenone*	21	9	1.46	1.27	85.3	80.3
Controls	23	9	9.90	6.46		
Progesterone*	19	9	1.80	1.72	78.9	77.1
Controls	24	13	8.52	7.51		
Testosterone*	17	8	2.28	2.25	73.1	60.7
Controls	19	8	8.47	5.71		
Androstene-3, 17-dione*	15	9	2.27	2.18	71.1	72.1
Controls	17	14	8.02	7.82		
Compound S (Reichstein)	5	—	5.14	—	53.2	—
Controls	4	—	10.98	—		
Dehydroepiandrosterone*	9	6	5.79	5.42	41.9	23.8
Controls	9	9	9.97	7.11		
Cholesterol*	7	4	6.16	6.95	31.9	4.1
Controls	8	4	9.04	7.25		
Androstane-17β-ol-3-one*	4	—	7.50	—	31.7	—
Controls	4	—	10.98	—		
Androstene-3β, 17β-diol *trans**	7	4	6.16	9.00	29.8	0
Controls	7	4	8.77	7.25		
Δ^4-Pregnene-17α,21-diol-3,20-dione	6	—	7.18	—	29.2	—
Controls	8	—	10.14	—		
Compound F (Kendall)	4	—	6.65	—	28.5	—
Controls	4	—	9.30	—		
Oestrone*	6	3	6.23	6.70	21.6	7.6
Controls	8	4	7.95	7.25		
Δ^4-Pregnene-11β,17α,21-triol-3, 20-dione	6	—	8.03	—	20.8	—
Controls	8	—	10.14	—		
Androstene-3β,17α-diol *cis**	4	—	8.93	—	10.6	—
Controls	4	—	10.55	—		
Dihydrotestosterone propionate*	5	—	8.52	—	8.4	—
Controls	4	—	9.30	—		
Diethylstilboestrol sulphate*	7	—	9.80	—	3.4	—
Controls	8	—	10.14	—		
Thyroxine*	5	5	9.04	12.40	0	0
Controls	5	5	8.76	7.25		
Pregnane-17α,21-diol-3,11,20-trione	5	—	11.30	—	0	—
Controls	4	—	9.30	—		

TABLE II

STRUCTURAL ASPECTS OF STEROIDS CAUSING A HIGHLY SIGNIFICANT INHIBITION OF STRAINS A AND B, LISTED IN ORDER OF PERCENTAGE INHIBITION AGAINST STRAIN A

Steroid	3-Keto	Δ^4	3-Hydroxy	20-Keto	21-Hydroxy or acetate	17-Hydroxy	17-Keto	11-Keto	11-Hydroxy
Compound A acetate (Kendall)	+	+	−	+	+	−	−	+	−
Cortisone acetate	+	+	−	+	+	+	−	+	−
Desoxycorticosterone	+	+	−	+	+	−	−	−	−
Desoxycorticosterone acetate	+	+	−	+	+	−	−	−	−
Cholestenone	+	+	−	Side chain	−	−	−	−	−
Progesterone	+	+	−	+	−	−	−	−	−
Testosterone	+	+	−	−	−	+	−	−	−
Androstene-3,17-dione	+	+	−	−	−	−	+	−	−

TABLE III

STRUCTURAL ASPECTS OF STEROIDS NOT CAUSING A SIGNIFICANT INHIBITION OF STRAINS A AND B, LISTED IN ORDER OF PERCENTAGE INHIBITION AGAINST STRAIN A

Steroid	3-Keto	Δ^4	3-Hydroxy	20-Keto	21-Hydroxy or acetate	17-Hydroxy	17-Keto	11-Keto	11-Hydroxy
Compound S (Reichstein)	+	+	−	+	+	+	−	−	−
Dehydroepiandrosterone	−	−	+	−	−	−	+	−	−
Cholesterol	−	−	+	Side chain	−	−	−	−	−
Androstane-17β-ol-3-one	+	−	−	−	−	+	−	−	−
Androstene-3β,17β-diol *trans*	−	−	+	−	−	+	−	−	−
Δ^4-Pregnene-17α,21-diol-3,20-dione	+	+	−	+	+	+	−	−	−
Compound F (Kendall)	+	+	−	+	+	+	−	−	+
Oestrone	−	+	+	−	−	−	+	−	−
Δ^4-Pregnene-11β,17α,21-triol-3, 20-dione	+	+	−	+	+	+	−	−	+
Androstene-3β,17α-diol *cis*	−	−	+	−	−	+	−	−	−
Dihydrotestosterone propionate	+	−	−	−	−	Propionate	−	−	−
Pregnane-17α,21-diol-3,11,20-trione	+	−	−	+	+	+	−	+	−

Discussion

Those steroids causing highly significant inhibition (Table II) possess a 3-keto-Δ^4 configuration. In this group those possessing a 20-keto and 21-hydroxy or acetate group caused the greatest inhibition. Two, cortisone acetate and compound A acetate, also have an 11-keto group. None of these structures, however, are peculiar to steroids which cause inhibition of amoebae growth, as 4 out of the 12 steroids listed in Table III have the same structures (3-keto-Δ^4, 20-keto, 21-hydroxy). All of these compounds

also possess a 17-hydroxy group, and two have an 11-hydroxy group. However, cortisone acetate and testosterone (Table II) also have a 17-hydroxy or acetate group.

There is also no correlation between the degree of inhibition and physiological activity, i.e. cholestenone, a physiologically inactive compound causes a greater inhibition than physiologically active testosterone and oestrone. Of additional interest is the fact that diethylstilboestrol, a nonsteroid synthetic female sex oestrogen always caused a complete inhibition, while oestrone was relatively ineffective.

None of the compounds tested produced any effect on the growth of the free-living amoebae, indicating that the physiology of these amoebae is probably markedly different from that of *E. invadens*.

It was previously shown (23) that certain of the above steroids were capable of completely inhibiting the growth of *E. invadens* in the presence of *E. coli* and *S. marcescens*. These same steroids in the bacteria-free medium with fresh liver only caused an average of 70–90% growth inhibition. In contrast, amoebae in control cultures with the bacteria developed more luxuriantly than controls with fresh liver. Therefore, the liver may be reducing or antagonizing the toxicity of the steroids. It has been shown (38) that the liver is capable of partially inactivating certain steroids.

In other growth studies with steroids, it was demonstrated that some, but not all 3-keto-Δ^4 steroids were effective in inhibiting chick embryo development (40); in this case steroids having an 11-keto group were most effective. However, only three 11-keto-Δ^4 steroids were effective against mouse lymphosarcoma (40). In addition, 3-keto-Δ^4 steroids were also effective against oestrogen-induced fibroids in guinea pigs (8).

Additional experiments on growth-inhibition of amoebae using large numbers of steroids may help to indicate a correlation between the degree of inhibition and structural formulae, which is not now apparent.

At present it appears that the ability to inhibit growth of amoebae is a function of the individual steroid.

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References

1. ACKERT, J. E. and DEWHIRST, L. W. Resistance of fowls to parasitism affected by female sex hormone. J. Parasitol. 36 : Suppl. 16. 1950.
2. ADDIS, C. J. Experiments on the relation between sex hormones and the growth of tapeworms (*Hymenolepis diminuta*) in rats. J. Parasitol. 32 : 574. 1946.

3. BAUGHN, C. The effect of adrenalectomy on acquired resistance of mice to infection with *Trichinella spiralis*. J. Parasitol. 38 : Suppl. 19. 1952.
4. BAUGHN, C. The effect of adrenalectomy on natural resistance of mice to infection with *Trichinella spiralis*. J. Parasitol. 38 : Suppl. 19. 1952.
5. BECK, J. W. Effect of gonadectomy and gonadal hormones on singly established *Hymenolepis diminuta* in rats. Exptl. Parasitol. 1 : 109. 1952.
6. BECK, J. W. and CHANDLER, A. C. Experiments on the nutrition and host relations of *Hymenolepis diminuta* in white rats with special reference to vitamins and hormones. J. Parasitol. 36 : Suppl. 44. 1950.
7. BENNISON, B. E. and COATNEY, G. R. The sex of the host as a factor in *Plasmodium gallinaceum* infections in young chicks. Science, 107 : 147. 1948.
8. BRUZZONE, S., FUENZALIDA, F., IGLESIAS, R., and LIPSCHUTZ, A. Antifibromatogenic action of steroids, with special reference to pregnenolones. *In* Symposium on steroids in experimental and clinical practice, *edited by* A. White. The Blakiston Company, New York. 1951.
9. CAMPBELL, D. H. The effect of sex hormones on the normal resistance of rats to *Cysticercus crassicollis*. Science, 89 : 415. 1939.
10. CAMPBELL, D. H. and MELCHER, L. R. Relationship of sex factors to resistance against *Cysticercus crassicollis* in rats. J. Infectious Diseases, 66 : 184. 1940.
11. CHERNIN, E. The relapse phenomenon in *Leucocytozoon simondi* in domestic ducks. J. Parasitol. 36 : Suppl. 22. 1950.
12. CHERNIN, E. The relapse phenomenon in the *Leucocytozoon simondi* infection of the domestic duck. Am. J. Hyg. 56 : 101. 1952.
13. CLAPHAM, P. A. On a sex difference in the infection rate of birds with *Syngamus trachea*. J. Helminthol. 17 : 192. 1939.
14. CULBERTSON, J. T. and MOLOMUT, N. Infection with *T. lewisi* in the hypophysectomized rat. Proc. Soc. Exptl. Biol. Med. 39 : 28. 1938.
15. CURTIS, M. R., DUNNING, W. F., and BULLOCK, F. D. Genetic factors in relation to the etiology of malignant tumors. Am. J. Cancer, 17 : 894. 1933.
16. GROSS, S. J. Male hormones for the prevention of relapses in malaria. Indian Med. Gaz. 82 : 65. 1947.
17. GROSS, S. J. Progesterone in the treatment of malaria. Indian Med. Gaz. 84 : 537. 1949.
18. HAUSCHKA, T. S. Sex of host as a factor in Chagas' disease. J. Parasitol. 33 : 399. 1947.
19. HEGNER, R. W. and MACDOUGAL, M. S. Modifying the course of infection with bird malaria by changing the sugar content of the blood. Am. J. Hyg. 6 : 602. 1926.
20. JARPA, A., AGOSIN, M., CHRISTEN, R., and ATIAS, A. V. Ensayos de quimioterapia de la enfermedad de chagas experimental. 7. Cortisona y fosfato de pentaquina. Bol. Inf. Paras. Chilenas, 6 : 25. 1951.
21. KUPFERBERG, A. B. and JOHNSON, G. Physiology of bacteria-free culture of *Trichomonas vaginalis*. 6. Effects of female sex hormones on population. Proc. Soc. Exptl. Biol. Med. 48 : 516. 1941.
22. LARSH, J. E. The effect of thyroid and thiouracil on the natural resistance of mice to infection with *Hymenolepis*. J. Parasitol. 33 : Suppl. 24. 1947.
23. LESSER, E. Can. J. Comp. Med. Vet. Sci. In press.
24. LUONGO, M. A., REID, D. H., and WEISS, W. W. The effect of ACTH in trichinosis. New Engl. J. Med. 245 : 757. 1951.
25. MARMORSTON-GOTTESMAN, J. and PERLA, D. The effect of bilateral suprarenalectomy in adult albino rats on the natural and acquired resistance to *Bartonella muris* anemia. J. Exptl. Med. 55 : 109. 1932.
26. MARMORSTON-GOTTESMAN, J., PERLA, D., and VORZIMER, J. Immunological studies in relation to the suprarenal gland. 6*a*. *Trypanosoma lewisi* infection in suprarenalectomized adult albino rats. J. Exptl. Med. 52 : 587. 1930.
27. NEGHME, A., BADINEZ, O., JARPA, A., THIERMANN, E., AGOSIN, M., and CHRISTEN, R. Acción de la cortisona sobre la trypanosomoses chagisica experimental del ratón suprarrenoprivo y total. Bol. Inf. Paras. Chilenas, 7 : 4. 1952.
28. OVERMAN, R. R., BASS, A. C., DAVIS, A. K., and GOLDEN, A. The effect of lipo-adrenal extract on ionic balance in fatal simian malaria. Am. J. Clin. Path. 19 : 907. 1949.
29. PERLA, D. Hypophysis and resistance. Proc. Assoc. Research Nervous Mental Diseases, 17 : 471. 1936.
30. PERLA, D. Relation of hypophysis to spleen. 3. Hypophysectomy and resistance to *Trypanosoma lewisi*. Proc. Soc. Exptl. Biol. Med. 40 : 91. 1939.

31. PERLA, D. and MARMORSTON-GOTTESMAN, J. Further studies on *T. lewisi* infections in albino rats and the protective action of splenic autotransplants. 2. The effect of thymectomy and bilateral gonadectomy on *T. lewisi* in albino rats. J. Exptl. Med. 52 : 601. 1930.

32. PICK, F. Sur un nouveau milieu de culture de l'amibe dysentérique comportant le presence de l'insuline. Bull soc. path. exotique, 39 : 345. 1946.

33. PICK, F. La longévité de l'amibe dysentérique sur le milieu a l'insuline. Compt. rend. soc. biol. 140 : 238. 1946.

34. REDMOND, W. B. Influence of cortisone on natural course of malaria in the pigeon. Proc. Soc. Exptl. Biol. Med. 79 : 258. 1952.

35. ROSEN, E. Cortisone treatment of trichinosis. Am. J. Med. Sci. 223 : 16. 1952.

36. SADUN, E. H. Relation of the gonadal hormones to the natural resistance of chickens and to the growth of the nematode, *Ascaridia galli*. J. Parasitol. 34 : Suppl. 18. 1948.

37. SADUN, E. H. Gonadal hormones in experimental *Ascaridia galli* infection in chickens. Exptl. Parasitol. 1 : 70. 1951.

38. SAMUELS, L. T. and WEST, C. D. The intermediary metabolism of the non-benzenoid steroid hormones. Vitamins and hormones. *Edited by* R. S. Harris, G. F. Marrian, and K. V. Thimann. Academic Press Inc., New York. 1952.

39. SCHMIDT, L. H. and SQUIRES, W. L. The influence of cortisone on primate malaria. J. Exptl. Med. 94 : 501. 1951.

40. STOCK, C. C., KARNOFSKY, D. A., and SUGIURA, K. Studies of steroids for inhibition of normal and abnormal growth in experimental animals. *In* Symposium on steroids. in experimental and clinical practice. *Edited by* A. White. The Blakiston Company, New York. 1951.

41. TRAGER, W. The resistance of egg-laying ducks to infection by the malaria parasite *Plasmodium lophurae*. J. Parasitol. 34 : 389. 1948.

42. TODD, A. C. Thyroid condition of chickens and the development of parasitic nematodes. J. Parasitol. 35 : 255. 1949.

43. TODD, A. C. and CROWDUS, D. H. Methyl testosterone in the diet of chicks and growth of the nematode *Ascaridia galli*. J. Parasitol. 37 : 322. 1951.

44. WOLF, A., KABAT, E. A., BEZER, A. E., and FONSECA, J. R. C. Activation of trypanosomiasis in rhesus monkeys by cortisone. Federation Proc. 10 : 375. 1951.

STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

IX. *DIDELPHONEMA*, A NEW GENUS OF NEMATODE FROM MARSUPIALS[1]

By R. W. Wolfgang[2]

Abstract

The spirurid nematode *Spirocerca cylicola* recorded by the author from the Trinidad opossum is regarded as a synonym of *S. longispiculata* Hill, 1939, which is made the type of a new genus, *Didelphonema*.

In 1939 Hill (4) described *Spirocerca longispiculata* from Virginia opossum (*Didelphis marsupialis virginiana*), from Oklahoma. In 1951, the author (5) described what he believed to be a closely related species from the insular opossum (*Didelphis marsupialis insularis*) from Trinidad. A re-examination of the author's specimens and a comparison of them with Hill's (the latter kindly loaned from the Helminthological Collection of the United States National Museum, through the courtesy of Dr. E. W. Price) show specimens of both lots to be conspecific. Thus, *Spirocerca cylicola* Wolfgang, 1951, becomes a synonym of *S. longispiculata* Hill, 1939.

The re-study of *S. longispiculata* shows that the gubernaculum which Hill believed to be absent is actually present, although somewhat transparent. The female tail is similar in length in both worms and the vulva in Hill's material is identical with that described by the present author for *S. cylicola*. These are the discrepancies in the two descriptions which have been resolved by comparison and re-examination of the materials.

Hill (4), in discussing *S. longispiculata*, gives the following reasons for differentiating his species from others in the genus:

> "The new species is seemingly not closely related to any of the other species in the genus, differing apparently from all of them in having: (1) definite pseudolabia, (2) recognizable dorso-dorsal and ventro-ventral external cephalic papillae, (3) an exceptionally long mesostome, (4) the cervical papillae in markedly asymmetrical arrangements, (5) *no accessory piece observed*, (6) paired caudal papillae of the male pedunculated, (7) dorsal transverse wrinkles on the caudal alae of the male, (8) a prominent postero-terminal protuberance on the female, (9) not been found in cysts or tumors and (10) smaller size."

He also lists differences from *S. lupi*, the type species of the genus, as follows:

> "(1) divergent uteri, (2) vulva near the middle of body, (3) longer and narrower stoma."

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

[2] *Research Assistant.*

At least five of the above-mentioned differences seem to the author to constitute sufficient evidence for separating *S. longispiculata* from the genus *Spirocerca*. These are:

(1) Definite pseudolabia,
(2) recognizable dorsodorsal and ventroventral papillae,
(3) the long slender mesostome which is cylindrical and not hexagonal as in *Spirocerca*,
(4) The vulva near the middle of the body, which is at variance with the generic diagnosis of *Spirocerca*, and
(5) worms not found in tumors or cysts as are those of *Spirocerca*.

Furthermore, the "dents" referred to by Hill actually represent a subprostomal extension of the chitinose mesostome. This chitinose extension is a complete ring (See Wolfgang (5, p. 366)) and not formed of hexagonal projections as in species of the genus *Spirocerca*.

Members of the genus *Spirocerca* are characterized as occurring in tumors and cysts in the stomach and oesophagus of carnivores, with the exception of *S. heydoni* which occurs in an Australian marsupial. Further significance of the host distribution appears to be related to previous suggestions by Cameron (1, 2), that not only are Australian and American marsupials probably not related to one another evolutionarily but that there is considerable evidence supporting the separation of the two groups taxonomically due to the differences in their helminth fauna. The present species is confined in its range to North America and the Lesser Antilles, and to a single host genus, namely *Didelphis*.

In view of the differences mentioned above, the erection of a new genus is suggested for which the name *Didelphonema* is proposed.

Didelphonema gen. nov.

These nematodes are of small to medium size. The anterior end is attenuated and terminates in a shallow oval stoma followed by an internal chitinous expansion leading into a long, slender mesostome which unites with the oesophagus. The oesophagus is unequally divided, the anterior portion being the shorter. The head bears two pseudolabia and four sets of double papillae on cuticular pulps, the dorsodorsals and ventroventrals are reduced but distinct. The cervical papillae are asymetrically arranged. In the female the tail bears papillae, the vulva divides the body into two equal parts, the gonads are amphidelphic, and the eggs are embryonated when laid. The male tail is coiled, bearing alae; there are four pairs of preanal, two pairs of postanal large pedunculated papillae, and five pairs of small terminal papillae all pedunculated; a gubernaculum is present; spicules are unequal.

Genotype: *Didelphonema longispiculata* (Hill, 1939) from the stomach and intestine of didelphin marsupials.

Synonyms: *Spirocerca longispiculata* Hill, 1939,
S. cylicola Wolfgang, 1951.

Discussion

Didelphonema fits best into the family Thelaziidae Railliet, 1916, and the subfamily Spirocercinae Chitwood and Wehr, 1932. Two characters listed in the generic diagnosis are at variance with the subfamily diagnosis as it now stands: the stoma is cylindrical and there are pseudolabia present around the mouth. Consequently, if the Spirocercinae is to contain *Didelphonema*, it will have to be modified to read: "stoma cylindrical or not and pseudolabia present or not". Other details are as given by Chitwood and Wehr (3).

References

1. CAMERON, T. W. M. Parasitology and evolution. Trans. Roy. Soc. Can., V, 44(3) : 1–20. 1950.
2. CAMERON, T. W. M. Parasitology, evolution and phylogeny. Endeavour, 11 : 193–199. 1952.
3. CHITWOOD, B. G. and WEHR, E. E. The value of cephalic structures as characters in nematode classification, with special reference to the super-family Spiruroidea. Z. Parasitenk. 7 : 273–335. 1935.
4. HILL, W. C. *Spirocerca longispiculata* n. sp. Am. Midland Naturalist, 21 : 636–640. 1939.
5. WOLFGANG, R. W. Studies on the endoparasitic fauna of Trinidad mammals. VIII. Parasites of marsupials. Can. J. Zool. 29 : 352–373. 1951.

ON THE LIFE HISTORY AND BIOLOGY OF *NOTOCOTYLUS STAGNICOLAE* HERBER, 1942 (FAMILY NOTOCOTYLIDAE)[1]

By Liang-Yu Wu[2]

Abstract

The adult and some of the larval stages of *Notocotylus stagnicolae* Herber, 1942 (family Notocotylidae) are redescribed and some details of their biology given.

Introduction

During the summer of 1951 a monostome cercaria was found in *Stagnicola palustris* collected from the Ottawa River.

Experimental infections were attempted using two young white mice, two young hamsters, and 10 one-to-seven-day-old chickens. Mice and hamsters gave negative results. Adults were recovered from chickens but the number of parasites from any bird was never very large, 10 parasites being the largest number recovered from a chick fed with 120 cysts. The caeca of the parasites were filled with red blood cells, which gave the digestive tracts of the parasites a bright red color. The parasites apparently fed on the host tissues rather than on the contents of the lumen.

The trematode was identified as *Notocotylus stagnicolae* Herber, 1942 (1) although it differs from it in some minor characters. Because of these differences the writer considers it desirable to describe the adult as well as some of the larval stages.

Materials and Methods

The infected snails were kept in separate containers. Some of the emerging cercariae were removed immediately from the water and fixed in hot formalin for measurement. Morphological studies were, however, made with living specimens.

Snails were kept individually in Petri dishes from nine in the morning until noon; encystment took place during this period on the wall of the container. The Petri dishes were then placed in larger containers in which water, which was changed daily, was kept. The cysts were later removed with a needle for experimental feeding.

The cercariae were also removed immediately after emergence to slides where encystment would take place. The slides were then fixed and mounted.

The adults recovered from chickens were fixed in Bouin and stained with borax carmine and alum carmine.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.
[2] *Graduate Assistant.*

Adult

The body is elongated, flattened, and concave ventrally (Fig. 1). The ventral surface is covered with spines. Those on the anterior end are scalelike (Fig. 3), and gradually decrease in size toward the middle of the body. The spines on the posterior half of the body are very fine and were seen up to the testicular region. Fine spines were also seen on the anterior half of the dorsal surface.

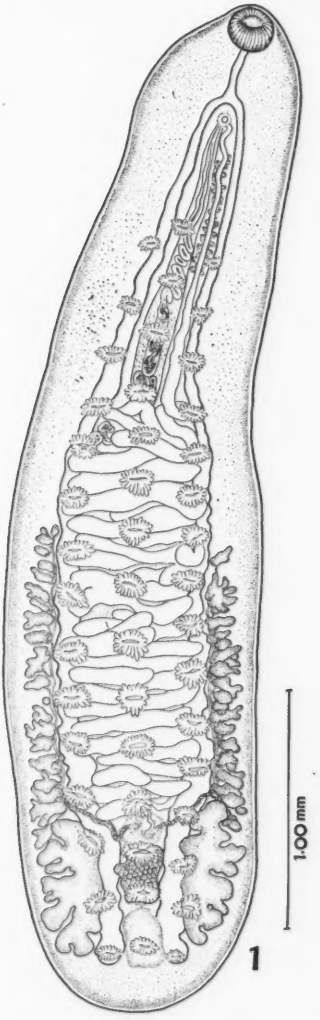

FIG. 1. *Notocotylus stagnicolae* Herber, 1942.

There are three rows of glands on the ventral surface. In 19 specimens examined, the number of glands in the lateral rows varied from 16 to 19 (usually 16 to 17), and in the middle row, from 14 to 16 (usually 15). The posterior gland in each row is smaller than those in front of it and the anterior gland in the lateral row is sometimes very small. The ventral glands are irregularly oval with a transverse slit and leaflike indentations. There is almost always one gland (in a few instances, two glands) in the middle row anterior to the most anterior glands in the lateral rows. The body in general differs from that described by Herber only in details. The specimens are larger

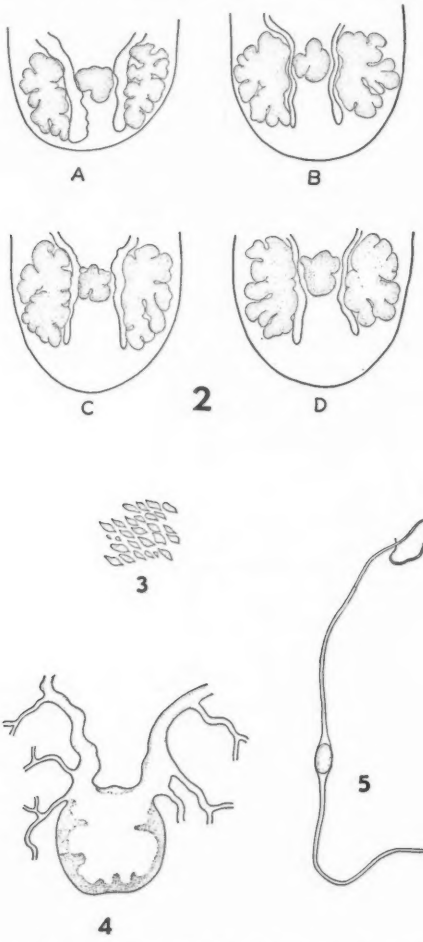

FIG. 2. (A–D). Posterior ends of adults showing the ovaries, testes, and caeca. FIG. 3. Scales from the anterior end, ventral surface. FIG. 4. Excretory bladder and the basal portions of two common collecting tubes. FIG. 5. An egg.

than Herber's (0.95 to 1.10 mm. wide by 3.89 to 4.32 mm. long) but they are also older (21 to 31 days instead of 13). The ovary is, however, smaller, but the testes are larger than in the type and each has from six to 11 lateral lobes along the outer margins (Fig. 2, A–D). The cirrus is very long and protrusible and covered thickly with conspicuous tubercles.

The uterus passes forward in numerous, closely packed loops which usually do not extend beyond the outer margins of the caeca. On a few occasions, extracaecal loops were seen. There are five to eight uterine loops in front of the vitellaria.

The excretory bladder is immediately behind the ovary and at a short distance from the posterior end. It receives two common lateral collecting tubes at its anterior corners. These tubes receive, in turn, many lateral branches on their outer margin (Fig. 4). The collecting tubes extend to the region of the oesophagus where they unite. The excretory pore is subterminal.

The eggs are oval, each usually having a long polar filament at each end (Fig. 5). Variations were observed on a few occasions. In one case, four filaments were seen at one pole with one at the other pole. On another egg, two filaments were seen on one pole, but none on the other. Twenty-four eggs from the anterior end of the uterus were fixed in formalin. They vary in size from 22 to 25 μ by 11 to 13 μ; the filaments vary in length from 200 to 280 μ.

Cercaria

Nine cercariae (Fig. 6), fixed in formalin and mounted in balsam, varied from 0.139 to 0.227 mm. and 0.306 to 0.515 mm. long. The tail varied from 0.038 to 0.060 mm. wide and 0.511 to 0.856 mm. long.

The oral sucker is muscular. At the caudal end there are two short, blunt, tubelike sucking organs for attachment ('locomotive pockets'). There are three eye spots, the median of which is anterior. The excretory system consists of two collecting tubes which unite behind the median eye spot with a short projection formed from the union. Therefore, the cercaria should belong to the Faust's *Yenchenyensis* group as suggested by Rothschild (2). These collecting tubes are filled with coarse granules of various sizes. Posteriorly, the collecting tubes empty into the excretory bladder at the anterior corners. The bladder is more or less square and opens posteriorly.

Cercariae began to emerge from the snail host between 10 o'clock in the morning and noon, leaving one after another at short intervals. They progress by whipping the tail in a figure-of-eight, and appear to be positively phototropic, because the encystment usually took place on the lighted side of the container. The time between emergence and encystment on slides is from two or three minutes to one-and-a-half hours; in a container, encystment takes place more promptly, usually in less than half-an-hour. Encystment took place on the wall of the container or on the shell of the snail.

The process of encystment was observed under the microscope. The cercaria rounded up and discharged a secretion rapidly from the body surface as

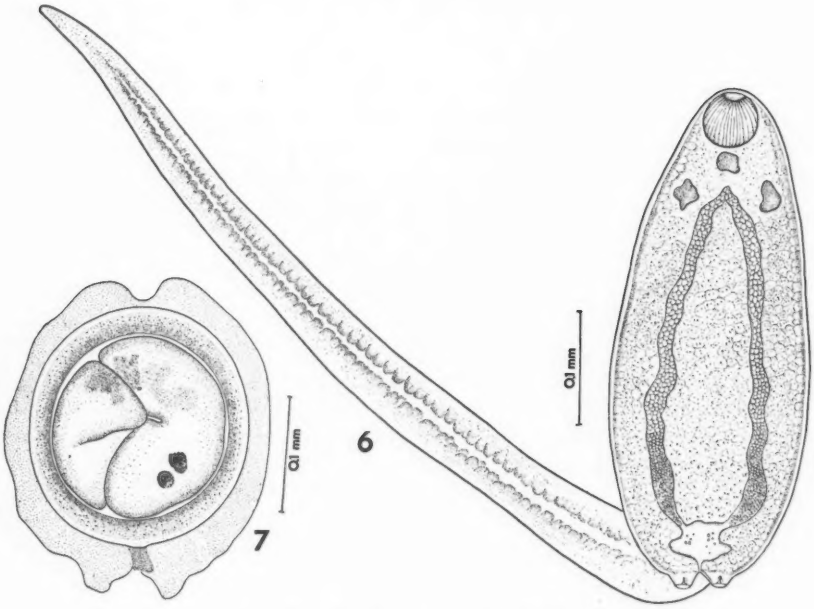

FIG. 6. A cercaria. FIG. 7. A metacercaria with its adhesive layer.

it retracted, leaving a notch indicating the anterior end of the cercaria (Fig. 7). This secretion formed a broad, thin, granular layer adhering to the substratum. In the meantime, the parasite began to rotate. The granules from the two main excretory tubes were being poured rapidly into the excretory bladder and subsequently discharged to the exterior as the cercaria rotated actively back and forth. These granules were dissolved in less than 10 min., so that a more transparent, thin outer layer of the cyst wall was formed. The rate at which these granules dissolved was gradually reduced as the process progressed; many were only partially dissolved and became embedded in the newly formed inner layer of the cyst wall. In addition to those more or less square granules from the excretory system, there were rod-shaped granules secreted from the parenchymatous cells of the body. They were also seen embedded in the newly formed inner cyst wall; the production of these rod-shaped granules has never been seen at the same time as the granules from the excretory system. These two kinds of granules together gave a brownish yellow color to the cyst wall. The maximum thickness of the cyst wall was formed in about half-an-hour and there was no increase of thickness seen during the hour the observation was continued. The rotation of the parasite and discharge of granules gradually slowed down at the end of the process.

Metacercaria

The metacercariae are subspherical (Fig. 7). Seventeen metacercariae, encysted on slides and subsequently stained and mounted, varied from 0.157 to 0.195 mm. by 0.167 to 0.198 mm. in diameter. This measurement includes only the real cyst wall; the outer adhesive layer is not included. The outer cyst wall is more transparent and thinner, being about 10 μ. This measurement was made on a cyst which had been under a cover glass during the whole process of cyst formation. The diameter of the cyst, including the real cyst wall, measured under such conditions, is much larger (0.253 mm.) than in uncompressed specimens.

While a number of small differences have been observed in all stages of this parasite, none seems to justify its separation from the species *N. stagnicolae*, and consequently it is referred to that species.

References

1. HERBER, E. C. Life history studies on two trematodes of the subfamily Notocotylinae. J. Parasitol. 28 : 179–196. 1942.
2. ROTHSCHILD, M. Notes on the classification of cercariae of the superfamily Notocotyloidea (Trematoda), with special references to the excretory system. Novitates Zool. 61 : 75–83. 1938.

A COMPARISON OF SOME SEASONAL AND TEMPERATURE-INDUCED CHANGES IN *PEROMYSCUS*: COLD RESISTANCE, METABOLISM, AND PELAGE INSULATION[1]

By J. S. Hart and O. Heroux

Abstract

The cold resistance of deer mice captured during the winter was greater than that of summer mice and was accompanied by a greater pelage insulation and a lower oxygen consumption at 1 – 2° C. On the other hand, the enhanced cold resistance of deer mice previously acclimated to cold was not accompanied by differences in pelage insulation. Moreover, in white-footed mice previously acclimated to different temperatures, oxygen consumption was similar at 1 – 2° C. These data indicate that the seasonal changes observed in *Peromyscus* are not identical to changes produced by acclimation in the laboratory. Environmental factors responsible for these differences are discussed.

Introduction

Acclimation of deer mice and white-footed mice to lower temperatures is associated with increase in cold resistance (5, 15). The increased resistance of white-footed mice results from enhanced ability to maintain high metabolic rates at low temperatures rather than from reduction in heat loss (6). In deer mice no change in pelage insulation was found when they were acclimated to high and low temperatures (5).

Little is known concerning the applicability of laboratory results on acclimation of mice, to mice in their natural environment. In nature the animals are subjected to many factors besides temperature, and the effects of temperature may be entirely different from those noted under laboratory conditions. The term "acclimatization" has therefore been used to signify physiological adjustment to changed environmental conditions in nature, as distinct from "acclimation" (5) which refers to single factor changes in the laboratory. The present investigation was undertaken to determine whether seasonal acclimatization in deer mice resulted in changes in cold resistance, metabolism, and pelage insulation comparable to those observed (5) during thermal acclimation.

Methods

Cold Resistance

Wild deer mice, *Peromyscus maniculatus gracilis* (Le Conte), were caught from July 31 to Aug. 14, 1951, in a wooded area belonging to the N.R.C. Montreal Road Laboratories in Ottawa and from February 11 to March 5, 1952, on farms near Kemptville, Ont. Summer mice were held for 24 hr., with food and water, at approximately 22° C. before cold resistance tests were carried out. Winter mice were similarly treated except that metabolism measurements were made on the day of capture and the mice were held at 1° C.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as N.R.C. No. 3055.

Cold resistance was determined by subjecting groups of two to seven mice to a range of temperatures (−9° to −16.5° C. for summer mice and −25° to −40° C. for winter mice) and measuring the time to death for each mouse. These temperatures were chosen to ensure death of mice in both series after periods ranging from 30 to 8000 min. Food was provided at all times. The procedure used was identical to that described in a separate communication (5).

Metabolism

Oxygen consumption of summer mice (caught from May 29 to June 18 and from Aug. 12 to 19, 1952, on the Montreal Road property of the National Research Council at Ottawa, Ont.) and winter mice (caught at Kemptville, Ont. in February and March 1952) was determined during the afternoon of the day of capture by a system previously described (4). Observations were started about one hour after the mice were placed in the metabolism chambers. Readings of oxygen uptake were made at five-minute intervals for about one to one and one-half hours, and all periods of visible activity were excluded from the average. The temperature of the chambers was regulated by a liquid bath held at 0 ± 0.1° C. The heat production of the mice raised the temperatures in the chamber to 1–2° C.

Pelage Insulation

After determination of oxygen consumption and cold resistance, the mice were skinned and the pelts stored at −20° C. until measurements were made. Pelage insulation was determined by a method previously described (5) except that the pelts were not extracted. For the most part, pelage insulation and oxygen consumption measurements were carried out on the same mice.

Results

Appearance of Summer and Winter Mice

The only apparent difference between summer and winter mice was in the pelage, which was visibly thinner in the former. No evidence was found in winter mice of the frostbite, shrivelled ears, or 'patchy' fur that were found in cold acclimated mice (5).

Cold Resistance

Winter mice were much more resistant to low temperatures than summer mice (Fig. 1). The differences were so great that survival times could not be compared at the same temperatures. At comparable survival times, the temperatures producing death (resistance temperatures) were about 20° C. lower for winter than for summer mice. Individual variability appeared to be somewhat greater than that described for acclimated mice (5); a phenomenon particularly noticeable in winter mice tested at −27° C. In both winter and summer mice the relation between temperature and log time to death appeared to be linear.

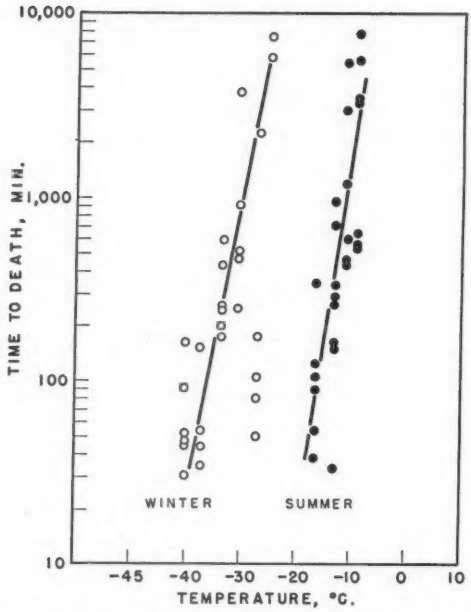

FIG. 1. Cold resistance curves for summer and winter mice. ●, Summer mice; ○, winter mice.

Metabolism

Oxygen consumption at temperatures of 1–2° C. was significantly lower in winter than in summer deer mice (Table I). The effect of body weight on oxygen consumption of summer and winter mice is shown in Fig. 2. It may be seen that weight had no appreciable influence on oxygen consumption and also that oxygen consumption of summer animals ranged higher than that of winter animals at any body weight. It is therefore concluded that the seasonal change in metabolism was not related to the minor seasonal differences in body weight (Table I). Furthermore, it is apparent that body weight differences in these mice may be virtually ignored in the calculation of oxygen consumption.

Pelage Insulation and Its Relation to Metabolism

Pelage insulation was significantly greater in winter than in summer deer mice and appeared to vary inversely with metabolic rate (Table I). Statistical examination of the insulation and metabolism of each mouse showed that these two characteristics were indeed inversely related during both winter and summer (Fig. 3). However, summer mice had a higher consumption rate than winter mice for equivalent insulation. Therefore the greater insulation of winter mice was not the only factor responsible for their lower rate of oxygen consumption.

TABLE I

COMPARISON OF SEASONAL AND TEMPERATURE INDUCED CHANGES IN OXYGEN CONSUMPTION AND PELAGE INSULATION OF *Peromyscus*

	Average body weight, gm.	Average oxygen consumption, at 1–2° C., ml./mouse/hr.	Average pelage insulation, °C./cal./hr./m.²
Acclimatized mice*			
Summer mice	21.26	185.4 ± 2.6	.180 ± .006
Winter mice	19.72	165.6 ± 2.6	.228 ± .007
Difference			
Absolute	1.54	19.8 ± 3.7***	.048 ± .009***
Acclimated mice**			
30° C.		204 ± 5	.146 ± .006
10° C.		201 ± 5	.156 ± .009

* *Data for P. maniculatus gracilis; 20 summer and 24 winter mice.*
** *Oxygen consumption data for P. leucopus noveboracensis (6).*
Pelage insulation data for P. maniculatus gracilis (5).
*** *Significant, $P < .001$.*

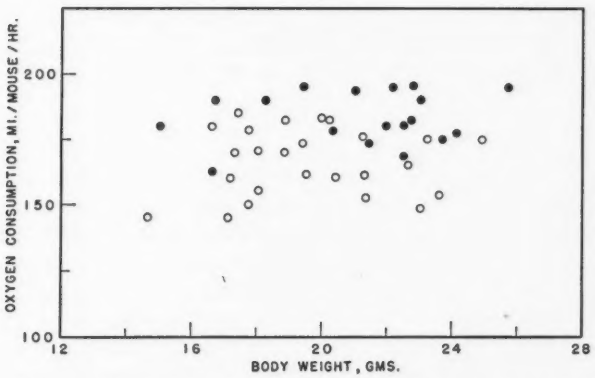

FIG. 2. Oxygen consumption at 1 – 2° C. in relation to body weight of summer and winter mice. ●, Summer mice; ○, winter mice.

The slope of the curve (Fig. 3) for winter mice was slightly, but not significantly, greater than that for summer mice. The relevant equations are:

$$\text{Winter:}\quad C = 202.8 - 153.7\,I$$
$$\text{Summer:}\quad C = 214.2 - 153.7\,I$$

where C is oxygen consumption in milliliters per hour and I is the pelage insulation in °C./cal./hr./m.².*

* *The standard deviation of an estimate of oxygen consumption at any level of I was 11.4, corresponding to a coefficient of variation of 6.5 at the mean level of consumption.*

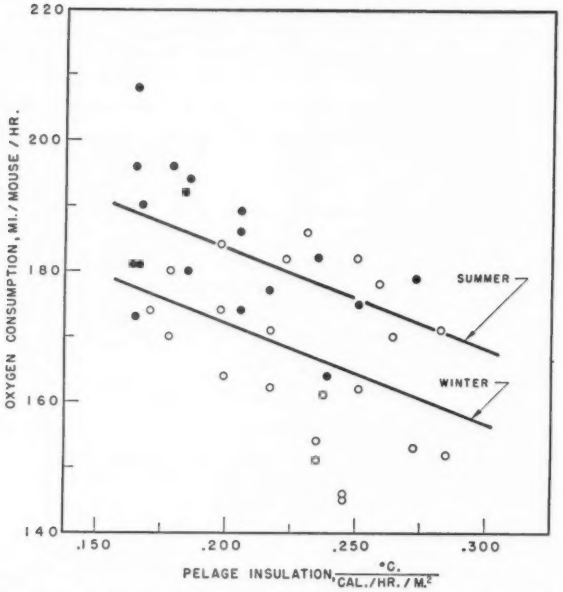

FIG. 3. Relations between oxygen consumption at 1 – 2° C. and pelage insulation of summer and winter mice. ●, Summer mice; ○, winter mice.

Discussion

The large seasonal change in cold resistance of deer mice indicates that acclimation phenomena observed in the laboratory actually have their counterpart in nature. Sealander (15, 16), using different methods, has recently found seasonal changes similar in direction to those reported here. From analogy with results on acclimation (5), it seems that summer mice were roughly equivalent in cold resistance to mice acclimated to 25° C., and winter mice had a resistance equivalent to mice acclimated to 0° C. (5)—a difference of 25° C. Thus, in spite of habits such as nest building and huddling (8, 16), which insulate the mice against temperature changes, these seasonal differences in cold resistance suggest that the mice were actually exposed to much lower temperatures during the winter than during the summer.

For equivalent cold resistance, winter mice as judged by appearance, seemed to be in better condition than acclimated mice. The increase in cold resistance acquired by mice during the winter was not accompanied by any of the visible lesions indicative of tissue damage due to cold (11), such as necrosis of ears and tails, that were frequently noted in mice acclimated to 0° C. (5). In acclimatized mice the fur appeared thicker than that of acclimated mice and oily deposits that caused clumping of the hairs were lacking.*

* *Clumping of the hairs caused decrease in pelage insulation of some of the acclimated mice. This condition was exaggerated by prolonged storage of the pelts at low temperatures. These pelts were therefore extracted before measurements were made.*

Exposure to fluctuating temperatures in nature as opposed to prolonged continuous exposure to a fixed temperature in the laboratory may have an important bearing on some of these differences.

Acclimatization to winter conditions was associated with a small but highly significant increase in pelage insulation. This phenomenon is considered to be of general occurrence among birds and mammals but actual data are limited. White-footed mice (15) and rabbits (13) have been shown to have a greater pelt weight and deer mice (9) have denser fur during winter than during summer. Increases in pelt weight have also been noted in animals acclimated to low temperatures (7, 15). However, in contrast to acclimatized animals, deer mice acclimated to low temperatures did not develop a significant increase in pelage insulation (Table I).

Increase in pelage insulation is only one of the ways in which over-all body insulation may be increased and heat loss at a given temperature reduced. The cumulative effect of insulative changes may be roughly determined by measuring heat production at a given temperature. The oxygen uptake at 1–2° C., which can be considered an index of heat production, was significantly reduced in winter mice, indicating an increase in over-all body insulation. Since the increased pelage insulation was not sufficient to account for the reduction in oxygen consumption, it is necessary to assume that other factors such as greater physiological insulation (lower skin temperatures) or lower body temperature (8) were operative in winter mice during the actual tests.

Reduction in oxygen consumption at a given temperature has been reported for other species during the winter (12, 13, 14). On the other hand, this metabolic phenomenon has not been demonstrated in animals acclimated to low temperatures (1, 2, 3, 10, 17). Similarly, in cold acclimated white-footed mice, there was no reduction in oxygen consumption at a given temperature (Table I). Full development of these metabolic and insulative changes may not occur in the laboratory unless conditions similar to those in nature are imposed (long term tests, fluctuating temperatures, changes in light etc.)

In an earlier communication (6) it was indicated that the increased cold resistance of mice acclimated to low temperatures could be explained largely by their enhanced ability to maintain greatly elevated metabolic rates for long periods of time. The increased cold resistance of winter mice is presumably associated with a similar mechanism, but they appear to have the additional advantage of reduced heat loss (lower oxygen consumption) in the cold, and therefore can survive at lower temperatures than summer mice for the same energy expenditure.

The seasonal changes discussed above indicate that acclimatization of animals in nature may not be an identical process to acclimation in the laboratory. This is not surprising in view of the multiplicity of factors acting on animals in their natural environment.

Acknowledgments

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References

1. ADOLPH, E. F. Oxygen consumptions of hypothermic rats and acclimatization to cold. Am. J. Physiol. 161 : 359–373. 1950.
2. GELINEO, S. Influence du milieu thermique d'adaptation sur la thermogénèse des homéothermes. Ann. physiol. physicochim. biol. 10 : 1083–1115. 1934.
3. GELINEO, S. Contribution to the study of heat production in the rabbit *Cuniculus cuniculus*. (In Serbian.) Glas Serbe. Acad. Sci. 192 : 181–199. 1949.
4. HART, J. S. Interrelations of daily metabolic cycle, activity, and environmental temperature of mice. Can. J. Research, D, 28 : 293–307. 1950.
5. HART, J. S. Relation between thermal history and cold resistance in certain species of rodents. Can. J. Zool. 31 : 80–98. 1953.
6. HART, J. S. Energy metabolism of the white-footed mouse, *Peromyscus leucopus noveboracensis*, after acclimation at various environmental temperatures. Can. J. Zool. 31 : 99–105. 1953.
7. HOESSLIN, H. V. Ueber die Ursache der scheinbaren Abhängigkeit des Umsatzes von Grösse der Körperoberfläche. Arch. Anat. u. Physiol., Physiol. Abt., pp. 323–379. 1888.
8. Howard, W. E. Relation between low temperature and available food to survival of small rodents. J. Mammal. 32 : 300–312. 1951.
9. HUESTIS, R. R. Seasonal pelage differences in *Peromyscus*. J. Mammal. 12 : 372–375. 1931.
10. KAYSER, CH. Échanges respiratoires des hibernants réveillés. Ann. physiol. physicochim. biol. 15 : 1087–1212. 1939.
11. LEBLOND, C. P. and DUGAL, L. P. Manifestations pathologiques produites par le froid au niveau des reins et des extrémités. Rev. can. biol. 2 : 542–545. 1943.
12. LEFÉVRE, J. Introduction critique à l'étude expérimentale du rayonnement aux diverses températures. J. physiol. 5 : 783–794. 1903.
13. MAYER, A. and NICHITA, G. Sur les variations du métabolisme du lapin après exposition au froid. Ann. physiol physicochim. biol. 5 : 621–632. 1929.
14. RIDDLE, O., SMITH, G. C., and BENEDICT, F. G. Seasonal and temperature factors and their determination in pigeons of percentage metabolism change per degree of temperature change. Am. J. Physiol. 107 : 333–342. 1934.
15. SEALANDER, J. A. Survival of *Peromyscus* in relation to environmental temperature and acclimation at high and low temperatures. Am. Midland Naturalist, 46 : 257–311. 1951.
16. SEALANDER, J. A. The relationship of nest protection and huddling to survival of *Peromyscus* at low temperatures. Ecology, 33 : 63–71. 1952.
17. SELLERS, E. A., REICHMAN, S., THOMAS, N., and YOU, S. S. Acclimatization to cold in rats: metabolic rates. Am. J. Physiol. 167 : 651–655. 1951.

LABORATORY STUDIES ON INSECT REPELLENCY[1]

By L. A. O. Roadhouse[2]

Abstract

The results of repellency tests conducted by Morton *et al.* on more than 4000 compounds were utilized to ascertain the effectiveness of typical molecular groups as repellents on the human skin. Other substances containing certain potentially repellent groups were then synthesized or prepared and subjected to repellency tests at Ottawa with the mosquito *Aedes aegypti* (L.) as the test insect. From the results of these tests, certain physical and chemical characteristics were evaluated in relation to repellency. The maximum repellent activity was invariably exhibited by compounds with atmospheric boiling points ranging from 230° to 260° C. It is presumed that chemicals with this range maintain a vapor concentration that is repellent to mosquitoes. The important atom from the standpoint of repellency seems to be oxygen. However, an oxygenated linkage such as hydroxy or carbonyl alone is not associated with repellency. Increased repellency appears to result when a hydroxyl group is once removed from unsaturation. It was confirmed that mixtures of substances enhance the repellent properties of various chemicals.

Introduction

The ultimate object of this investigation was the discovery of chemicals that might be easily synthesized from readily available raw materials and that would repel mosquitoes, black flies, and other biting insects. A logical approach to the problem was initially impossible because there was no adequate theory of the mechanism of repellent action. As a result substances could not be "designed" molecularly for repellent action. Recourse had first to be made to repellency tests of a random selection of typical substances in the hope that subsequent analysis of the results obtained would enable an evaluation of certain physical and chemical characteristics.

The original random selection of substances was greatly facilitated by the results of the repellent tests previously conducted by Morton *et al.* (5) on more than 4000 compounds using the human-arm-test method. The results obtained by Morton *et al.* were examined to discover whether certain functional groups are associated with insect repellency. In the classification of effectiveness used by these workers, a rating of "4" was applied to repellents that prevented biting for more than 180 min.; "3" to those effective for 121 to 180 min.; "2" to those effective for 61 to 120 min.; and "1" to those effective for 60 min. or less. However, Morton *et al.* did not give any systematized summary of their work.

To systematize the results of Morton *et al.*, it was necessary to calculate a value for the repellency of typical molecular groups. This was accomplished by first classifying the substances tested by these workers into typical chemical groups (e.g., monoalcohols, ethers, ketones, etc.) and then dividing the number of substances in each chemical group into the sum of the products obtained by

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multiplying the number at each rating by the corresponding rating. For example, of 196 substances in the monoalcohol group tested by Morton *et al.*, 30 had been rated at "4", 14 at "3", 52 at "2", and 100 at "1"; the value for the repellency of this chemical group was therefore

$$\frac{(30 \times 4) + (14 \times 3) + (52 \times 2) + (100 \times 1)}{196} = 1.87 .$$

This value may be termed a *class average.* The class averages for typical chemical groups tested by Morton *et al.*, as determined by the above method, are listed in Table I.

TABLE I

RELATIVE REPELLENCIES (HIGHEST FIRST) OF TYPICAL MOLECULAR GROUPS IN COMPOUNDS TESTED BY MORTON *et al.*

Group	Number tested	Class average*
Section 1 (more than 100 tested)		
Hydroxyethers	134	2.46
Glycols	112	2.30
Amides, imides, amide-esters	270	2.19
Hydroxyesters	221	2.16
Monoalcohols	196	1.87
Aldehydes, ketones, and hydroxy-ketones	254	1.78
Ether esters and epoxy esters	200	1.66
Nitriles	137	1.60
Esters and keto-esters	1600	1.46
Ethers	349	1.30
Hydrocarbons	110	1.28
Section 2 (50–100 tested)		
Nitrogenheterocyclic compounds	70	2.10
Acid anhydrides	50	2.08
Acetals	70	2.04
Amines	52	2.00
Organosulphur compounds	69	1.36
Natural oils	67	1.32
Section 3 (less than 50 tested)		
Oximes	15	2.53
Wood distillate	30	2.14

* *See text.*

In this table the chemical groups are divided into three sections according to the number of compounds tested. Only compounds having the chemical groups listed in Section 1 have been studied carefully in the present investigation.

An appraisal of the class averages obtained indicated that the presence and arrangement of certain groups of atoms were associated with insect repellency. In the present investigation, the most promising class averages of the groups tested in Section 1 were found to be of compounds that have the highest combined fat and water solubility, and that may associate in the liquid phase with themselves or with water in order to maintain a repellent concentration in the vapor phase for the longest time. On the basis of this information, further substances were prepared (Tables II to VII) and as those with favorable repellent properties were discovered the molecular patterns were altered with the object of enhancing the repellent action. Some of the factors that govern the effectiveness of chemicals as insect repellents are considered in this paper.

Testing Technique

In the study reported in this paper, the technique used in testing for repellency was a modification of that developed by Granett (2). Wire-mesh cages of approximately 9 cu. ft. capacity, containing 1000 *Aedes aegypti* (L.) mosquitoes of both sexes were kept in a room at 27° ± 1° C. and 70 ± 10% relative humidity. The mosquitoes were reared by the technique described by Trembley (6), with minor changes (3). No cage was used for repellency tests unless the biting rate exceeded 40 per minute on the untreated forearm. The forearm was then covered with 1 gm. of the candidate repellent and inserted into the cage for a three minute period. This was repeated at half-hour intervals. The time to the first, second, and third bites was recorded together with the number of mosquitoes landing during each three-minute period. The biting rate on an untreated arm was determined at the beginning of the trial period, after three hours, and again at the end of the trial period if the test continued longer than three hours. Each compound was tested on two to five subjects, one subject being the same individual throughout all the tests. The landing rates and times to bites are averages of these results.

Repellencies of Chemicals in Various Groups

Monoalcohols

The repellency times of the monoalcohols tested are shown in Table II.

It should be appreciated that thousands of compounds are mildly repellent, but only a limited number are strongly repellent. As Morton *et al.* (5) found, those monoalcohols that were least odoriferous to man repelled the mosquitoes. (The monoalcohols appear to include more compounds that are repellent to insects and have little or no odor to man than the other groups that Morton *et al.* investigated.) There was little to choose between primary, secondary, and tertiary alcohols. Apparently a maximum of repellency is associated with a boiling point of approximately 260° C. and alcohols containing a cyclic system of atoms are more repellent than acyclic ones.

TABLE II

REPELLENCY TIMES OF VARIOUS MONOALCOHOLS AND THEIR DERIVATIVES

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source*	Landing rate**	Time to bite, min.		
					First	Second	Third
132	Ethyl orthoacetate	142/—	E.K.	66***	0	0	0
158	Ethyl orthopropionate	150/—	E.K.	78***	0	0	0
58	Dimethylallyl carbonate	84/12	E.K.	45	30	30	30
37	Fenchyl alcohol	201/—	K.	13	30	30	30
19	1-Phenylethanol	204/—	E.K.	2	50	50	50
148	2,2,4,8,10,10-Hexamethyl-5-methylolundecane	210/—	R.H.	134	60	90	90
34	Borneol (in peanut oil)	210/—	B.D.H.	41***	0	0	0
193	Phenylisopropylcarbinyl acetate	212/—	T.W.	168	30	30	30
33	1-Menthol	215/—	B.D.H.	70	30	30	30
25	1-Phenylethyl acetate	215/—	T.W.	80	50	50	50
35	α-Terpineole	216/—	E.K.	22	105	105	105
113	(*a*) 2-Phenylethanol, pure	220/—	E.K.	6	160	160	160
205	(*b*) 2-Phenylethanol, 25% soln. in lauryl alcohol	—	T.W.	9	90	90	90
206	(*c*) 2-Phenylethanol, 25% soln. in glycerine	—	T.W.	38	30	30	30
207	(*d*) 2-Phenylethanol, 10% soln. in glycerine	—	T.W.	53	30	30	30
187	Phenylisopropylcarbinol	220/—	T.W.	16	150	150	150
197	2-Aminocyclopentanol	116/19	T.W.	108***	0	0	0
169	Linalyl acetate	220/—	K.	107	30	30	30
125	Benzyldimethylcarbinol	128/14	T.W.	22	120	120	120
148	(*a*) Geraniol, pure	230/—	K.	35	80	90	90
208	(*b*) Geraniol, 25% soln. in lauryl alcohol	—	T.W.	95	30	60	90
209	(*c*) Geraniol, 25% soln. in glycerine	230/—	K.	120***	0	0	0
210	(*d*) Geraniol, 5% soln. in glycerine	—	T.W.	144	0	30	30
168	Geraniol acetate	245/—	K.	142	90	90	90
50	Eugenol methyl ether	245/—	T.W.	129	60	60	90
110	Acetic acid ester of 2,2,4,8,10, 10-hexamethyl-5-methylolundecane	133/2	T.W.	158	0	0	0
203	2-N-*n*-butylaminocyclohexanol	155/7	T.W.	12	150	210	210
57	3,7-Dimethyl-3-acetoxyoctyne-1-ene-6	250/—	T.E.	240	45	45	45
104	Cinnamyl alcohol	257/—	E.K.	21	270	270	270
182	2-Phenylcyclohexanol (mixture of diastereomers, mostly *trans*)	(M.p. 54)	D.	69	180	210	210
105	Cinnamyl acetate	265/—	T.E.	136	45	135	165
198	*trans*-Acetylaminocyclopentanol	192/16	T.W.	130***	0	0	0
55	Cardanol	234/1	I.P.V.	183***	0	0	0

* *E.K., Eastman Kodak Company; K, Kahlbaum; R.H., Rohm and Haas Company; B.D.H., British Drug Houses; T.W., supplied by Prof. George F Wright, University of Toronto, Toronto, Ont.; T.E., Tennessee Eastman Company; D., Dow Chemical Company; I.P.V., Irvington Paint and Varnish Company.*

** *Number landing in three-minute period on treated arm.*

*** *At the beginning of the test; or else, after 30 min.*

The best repellent in this class proved to be cinnamyl alcohol, which was better than 2-phenylcyclohexanol. The latter compound is in use as a repellent, and was reported by Morton *et al.* (5) to be more effective than the former. If this difference in effect is due to some idiosyncrasy of the test subject, then the result reported by Morton *et al.* (5) is probably the more

reliable, since a larger number of individuals were used by those workers than in this study. However, the results in both laboratories for other compounds, such as alpha terpineol and geraniol, are essentially the same. There is a large difference in repellency between cinnamyl alcohol and its acetate, both of which are relatively high in repellent action. On the other hand, there is no appreciable difference between geraniol and its acetate, both of which are but slightly repellent.

The low repellency of geraniol suggested the examination of Ceylon oil of citronella, to ascertain the contribution which this alcohol made to the repellent activity of the oil. Tests of various fractions of the oil revealed that the activity was largely due to citronellol and not to the geraniol, which comprised a large portion of the oil. The original oil possessed a repellent time to the first bite of 90 min., whereas citronellol gave a corresponding time of 120 min. Pure geraniol was effective for 80 min.

Alpha Hydroxy Acid Esters

The repellency times of a series of alpha hydroxy, methoxy, and acetoxy esters are shown in Table III.

TABLE III

REPELLENCY TIMES OF VARIOUS α-HYDROXY ACID ESTERS AND THEIR DERIVATIVES

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source	Landing rate	Time to bite, min.		
					First	Second	Third
136	Ethyl glycolate	160/—	T.W.	75	30	30	30
27	*dl*-Lactic acid	122/15	E.K.	108*	0	0	0
121	Ethyl α-methoxyphenylacetate	235/—	T.W.	190	120	150	180
63	Methyl decanoate, α-hydroxy	226/—	T.W.	96	140	170	170
85	Methyl decanoate, α-acetoxy	102/2.5	T.W.	187*	15	15	15
82	Ethyl decanoate, α-hydroxy	128/16	T.W.	47	180	180	195
86	Ethyl decanoate, α-acetoxy	130/10	T.W.	132*	15	15	15
83	*n*-Propyl decanoate, α-hydroxy	134/11	T.W.	105	90	120	120
87	*n*-Propyl decanoate, α-acetoxy	141/10	T.W.	130*	30	30	30
84	*n*-Butyl decanoate, α-hydroxy	249/—	T.W.	99	105	120	120
88	*n*-Butyl decanoate, α-acetoxy	149/10	T.W.	126*	0	0	0
89	Benzyl decanoate, α-hydroxy	140/2	T.W.	26	90	90	90
90	Benzyl decanoate, α-acetoxy	160/5	T.W.	36	120	120	120
175	Benzyl glycolate	245/—	T.W.	9	180	210	210
18	Benzyl lactate	145/15	E.K.	10	360	370	390
91	Benzyl α-acetoxypropionate	130/3	T.W.	61	105	165	180
170	Ethyl mandelate	250/—	T.W.	5	300	300	300
173	Ethyl phenoxyacetate	251/—	T.W.	74	60	90	120
26	1-Phenylethyl lactate	265/—	T.W.	63	80	100	115
151	Benzyl mandelate	(M.p. 96)	T.W.	168*	0	0	0
111	Lactic acid ester of 2,2,4,8,10, 10-hexamethyl-5-methylolundecane	160/1	T.W.	132*	0	0	0
122	Propyl benzilate	220/35	T.W.	360*	0	0	0
123	*n*-Butyl benzilate	157/1	T.W.	240*	0	0	0
124	*n*-Amyl benzilate	231/26	T.W.	210*	0	0	0
184	*n*-Butyl citrate	300/—	E.K.	138*	0	0	0
157	Dibenzyl malate	(M.p. 42)	T.W.	75	30	60	90

** At the beginning of the test; or else, after 30 min.*

Ethyl alpha methoxyphenyl acetate is less repellent than its demethylated analogue, ethyl mandelate, a recognized repellent, and is also inferior to some of the other 2-hydroxy-4,6,6-trimethylheptanoic esters tested. The marked superiority of the hydroxy over the acetoxy methylester is consistent throughout the series. The effectiveness of the hydroxy esters rises to a maximum for the ethyl hydroxy ester and declines as the boiling points of the higher members of the homologous series rise above an estimated boiling point at atmospheric pressure of approximately 250° C. The last member, benzyl hydroxy decanoate, is a relatively poor repellent.

However, this decrease in activity is probably not due to the presence of the benzyl group. Benzyl glycollate and benzyl lactate give protection for more than three and six hours, respectively. The acetate ester of the lactate (benzyl alpha acetoxy propionate) is still fairly active despite the blockage of the hydroxyl group. The high repellent power of ethyl mandelate seems, in this series, to be related to a boiling point of approximately 250° C. Activity declines thereafter with increase in boiling point until no activity at all is observed with benzyl mandelate and octadecyl lactate.

These observations concerning boiling point should not imply that structural differences are insignificant. It is only necessary to contrast the effectiveness of the structurally isomeric ethyl phenoxyacetate with ethyl mandelate to recognize that structure plays a part in repellency. Nevertheless, compounds in this group that show maximum repellency invariably have atmospheric boiling points ranging from 230° to 260° C. Compounds with a boiling point in this range may be expected to give a vapor pressure likely to stimulate the insects' olfactory apparatus. A great many of the effective compounds contain groups that should be strongly associative at the temperature of the body.

1,3-Glycols

The results of tests on 1,3-glycols are listed in Table IV.

Since one of the commercially successful repellents is 2-ethylhexanediol-1,3, a series of 1,3-diols was investigated. The most effective of this series was the formal derivative of the relatively inactive 1-phenyl-1,3-propanediol, namely, 4-phenyl-1,3-dioxane.

Presumably the low activities of 1-phenyl-1,3-propanediol and its diacetate are due to their high boiling points. Formals such as 4-phenyl-1,3-dioxane are easily hydrolyzed under aqueous acidic conditions. One may speculate that the effectiveness of this compound is due to its favorable boiling point, which allows it to reach the site of olfactory perception more easily than the less volatile 1-phenyl-1,3-propanediol, to which it may revert on hydrolysis.

In view of the repellency of some cyclohexanols the activity of the predominant diastereomer of 1,3-cyclohexanediol was investigated. The *meso* isomer was entirely inactive, but this was not unexpected as it is a solid with a high melting point. On the other hand, its diesters, which are liquid,

TABLE IV

REPELLENCY TIMES OF VARIOUS 1,3-DIOLS AND THEIR DERIVATIVES

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source	Landing rate	Time to bite, min.		
					First	Second	Third
107	4-Phenyl-1,3-dioxane	130/17	T.W.	35	270	270	270
4	1,3-Cyclohexanediol (*d, l*)	(M.p. 107)	T.W.	90*	0	0	0
3	*Meso*-1,3-cyclohexanediol	(M.p. 84)	T.W.	60*	0	0	0
24	*Meso*-1,3-cyclohexanediol, *n*-butyral	90/10	T.W.	67	45	45	45
15	*Meso*-1,3-cyclohexanediol, diformate	114/8	T.W.	13	50	70	100
16	*Meso*-1,3-cyclohexanediol, diacetate	131/15	T.W.	69	60	70	70
17	*Meso*-1,3-cyclohexanediol, dipropionate	154/15	T.W.	31	180	220	240
21	*Meso*-1,3-cyclohexanediol, di-*i*-butyrate	140/8	T.W.	37	210	210	210
20	*Meso*-1,3-cyclohexanediol, di-*p*-butyrate	157/8	T.W.	65	30	40	50
22	*Meso*-1,3-cyclohexanediol, di-*n*-valerate	160/6	T.W.	130*	10	10	10
45	*Meso*-1,3-cyclohexanediol, dilactate	—	T.W.	125*	0	0	0
219	2,2,4-Trimethyl-1-acetoxy-3-hydroxy-pentane	232/—	T.E.	40	140	140	140
218	2,2,4-Trimethyl-1,3-diacetoxypentane	238/—	T.E.	75	30	60	60
227	2,2,4-Trimethyl-1,3-dihydroxypentane	90/5	T.E.	40	330	330	360
108	1-Phenyl-1,3-propanediol	165/10	T.E.	74	45	60	75
106	1-Phenyl-1,3-diacetoxypropane	286/—	T.E.	165	30	30	30
8	Aminotrimethylolmethane, triacetate	65/10	T.W.	108*	0	0	0
6	Aminotrimethylolmethane	(M.p. 165)	T.W.	162*	0	0	0
5	Nitrotrimethylolmethane	(M.p. 159)	T.W.	108*	0	0	0
1	Trimethylolethane	(M.p. 186)	T.W.	60*	0	0	0
2	Trimethylolpropane	(M.p. 59)	T.W.	120*	0	0	0
13	Tetramethylolcyclohexanol	(M.p. 131)	T.W.	78*	0	0	0
12	Tetramethylolcyclopentanol	(M.p. 96)	T.W.	90*	0	0	0
11	Tetramethylolcyclopentanone	(M.p. 143)	T.W.	120*	0	0	0

** At the beginning of the test; or else, after 30 min.*

showed activity that reached a maximum at the dipropionate and *di*-isobutyrate, which boiled at temperatures that would be approximately 265° C. at atmospheric pressure.

1,2-Glycols

The results for several 1,2-glycols are shown in Table V.

Pinacol and 2,3-butanediol, which have low boiling points, were relatively ineffective. One might have expected 2-methyl-1-phenylpropanediol-1,2 to be repellent, since its atmospheric boiling point is approximately 260° C. However, its repellency was almost negligible. It is a solid that melts at 63° C., and the boiling point is at the upper critical level for repellent action. It was also ineffective in solution in peanut oil. On the other hand, a mixture of three parts of it with one part of ethyl mandelate was as effective as the best repellent tested. The same effect was observed when it was mixed with an equal weight of piperonal. A similar enhancement of repellent activity was previously reported by Jackowski and Pijoan (4).

TABLE V

REPELLENCY TIMES OF VARIOUS POLYALCOHOLS AND THEIR DERIVATIVES

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source	Landing rate	Time to bite, min.		
					First	Second	Third
134	Pinacol	173/—	E.K.	56	75	90	90
131	*Levo*-butanediol-2,3	184/—	T.W.	108	0	15	30
135	*Levo*-butanediol-2,3 diacetate	200/—	T.W.	60	30	30	30
186	1-Phenyl-1,2-dimethoxypropane	224/—	T.W.	28	120	120	120
162	Butynediol-1,4	126/2	T.W.	87*	0	0	0
217	1,5-Pentanediol	133/14	T.W.	120*	0	0	0
223	2,2-Dimethylpentanediol-1,5	244/—	T.E.	221*	0	0	0
178	Diglycolmonorincinoleate	260/—	G.P.**	38*	0	0	0
153	2-Methyl-1-phenylpropanediol-1, 2; pure, m.p. 63	153/17	T.W.	56	0	30	60
155	2-Methyl-1-phenylpropanediol-1, 2; 66% mixture with peanut oil	—	T.W.	198	0	30	30
154	2-Methyl-1-phenylpropanediol-1, 2; 75% soln. in ethyl mandelate	—	T.W.	22	360	360	360
211	2-Methyl-1-phenylpropanediol-1, 2; 50% mixture with piperonal	—	T.W.	27	360	360	360
200	1-Methyl-1-phenylpropanediol-1,2	153/17	T.W.	11	330	330	330
216	3-Phenylpentanediol-2,3, mixed with rearrangement products	150/14	T.W.	35	150	150	150
220	3-Phenylpentanediol-2,3, mixed with 2-methyl-1-phenylpropanediol-1,2	—	T.W.	65	30	120	150
139	4,6,6-Trimethylheptanediol-1,2	160/12	T.W.	67	30	150	180
137	Diglycol laurate	280/—	G.P.	70	90	120	120
166	Glyceryl monorincinoleate	290/—	G.P.	213*	0	0	0
221	1,4-Diacetoxydecahydronaphthalene	295/—	T.E.	104	60	60	60
177	Triethyleneglycol di-2-ethylbutyrate	345/—	G.P.	60	60	60	60
191	Oleate of triethyleneglycol monomethyl ether	356/—	G.P.	156*	0	0	0
192	Oleate of triethyleneglycol monobutyl ether	380/—	G.P.	147*	0	0	0
228	1,6-Hexanediol	(M.p. 40)	T.E.	120*	0	0	0
229	1,10-Decanediol	(M.p. 73)	T.E.	123*	0	0	0
230	1-Hydroxycyclohexylphenylcarbinol	(M.p. 103)	T.E.	120*	0	0	0
161	Hexadiyne-2,4-diol-1,6	(M.p. 112)	T.W.	99*	0	0	0
—	2-Ethylhexanediol-1,3	—	T.W.	58	180	210	210

* *At the beginning of the test; or else, after 30 min.*
** *G.P., Glyco Products Incorporated.*

Isomeric 1-methyl-1-phenylpropanediol-1,2 (a liquid that boils at approximately 260° C.) is an extremely good repellent. The homologue 3-phenylpentanediol-1,2 is much less active, although the boiling points of the two compounds are nearly identical.

The compound 4,6,6-trimethylheptanediol-1,2 has approximately the same boiling point and the same number of carbon atoms as 1-methyl-1-phenylpropanediol-1,2, yet its repellency is much lower. It is very unlikely that this difference in effectiveness is due to the differing fat–water solubilities of the two compounds. Other comparisons encountered during this investigation suggest that the smell of a phenyl group is objectionable to insects.

Aldehydes and Ketones

The results obtained on this group of compounds are shown in Table VI.

TABLE VI

REPELLENCY TIMES OF VARIOUS ALDEHYDES AND KETONES

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source	Landing rate	Time to bite, min.		
					First	Second	Third
143	2,4-Pentanedione	140/—	T.W.	60*	0	0	0
130	Benzoin methyl ether	(M.p. 50)	T.W.	80*	0	0	0
40	Camphor in peanut oil	0.178/—	B.D.H.	115	30	30	30
36	Fenchone	190/—	T.W.	60	30	30	30
49	Citronellol	208/—	E.K.	48	30	30	30
156	3-Phenylbutanone-2	107/17	T.W.	132	30	30	30
56	2-Methoxy-2-methyl-1-phenyl-propanone-1	88/14	T.W.	180	45	45	45
194	2,2-Dimethyl-1-phenylpropanone-1	220/—	T.W.	52	30	30	30
167	α-Phenylisobutyraldehyde	110/14	T.W.	125	120	120	120
189	Hexahydro-4,4-endomethyleneindane-5-one	225/—	T.E.	17	60	60	60
732	Piperonal, 20% soln. in methanol	245/—	T.W.	6	130	135	180
213	Piperonal, 60% soln. in glycerol	—	T.W.	37	180	210	210
211	Piperonal, 50% mixture with 2-methyl-1-phenyl-propanediol-1,2	—	T.W.	27	360	360	360
196	2-Acetoxy-1-phenylpropanone-1	144/16	T.W.	58	30	30	30
195	2-Hydroxy-1-phenylpropanone-1	122/14	T.W.	18	330	330	360
201	1-Hydroxy-1-phenylpropanone-2	126/14	T.W.	9	360	360	360
159	2-Hydroxy-2-methyl-1-phenylpropanone-1	132/19	T.W.	5	210	210	210
52	2,2,7,7-Tetramethyl octanedione-3,6	(M.p. 19)	T.W.	179	40	40	40

** At the beginning of the test; or else, after 30 min.*

The rating for aldehydes and ketones determined by Morton *et al.* (5) and confirmed by this investigation is not high. Some of the aldehydes and ketones listed were tested only because they were intermediates in the synthesis of other compounds described in this paper. Most of them have boiling points below the range at which compounds have been found effective. The best of this group is piperonal, which boils at 245° C. Although the boiling point of 1,4-diketones is in the optimum temperature range, the repellency of this type of compound seems to be very low.

The rating of Morton *et al.* (5) for hydroxyketones was also not high, but in the present investigation such compounds appeared to be effective. This effectiveness does not seem to extend to compounds in which the hydroxy group is blocked by alkoxy and acetoxy groups (e.g., 2-methoxy-2-methyl-1-phenylpropanone-1 and 2-acetoxy-1-phenylpropanone-1) despite the ease with which such substituents can be hydrolyzed. The low rating for 2-acetoxy-1-phenylpropanone-1 is remarkable since it boils in the same range as 2-hydroxy-1-phenylpropanone-1, which is strongly repellent.

Miscellaneous Types

Compounds that do not fit into the previous classes are listed in Table VII.

TABLE VII

REPELLENCY TIMES OF MISCELLANEOUS COMPOUNDS

Code No., T—	Substance	Boiling point, ° C./ Pressure, mm. mercury	Source	Landing rate	Time to bite, min.		
					First	Second	Third
171	Acetin, mono	158/—	K.	82*	0	0	0
29	Aluminum powder	—	T.W.	108*	0	0	0
14	Bioxazoline	(M.p. 213)	T.W.	78*	0	0	0
46	Cineole-1,4	173/—	B.D.H.	150*	0	0	0
47	Cineole-1,8	177/—	B.D.H.	96*	0	0	0
222	Isopentenylacetoacetic acid, ethyl ester	225/—	T.E.	40	90	120	150
163	Ethyl coumalate	170/1.5	T.W.	240*	0	0	0
164	Methyl coumalate	(M.p. 68)	T.W.	117	0	0	0
224	Propyl coumalate, 80% mixture with ethyl mandelate	(M.p. 60)	T.W.	70	150	150	150
190	1-Cyclohexyl-1-carboethoxycyclohexane	273/—	T.E.	210*	0	0	0
53	Di-*t*-butylfuran	185/—	T.W.	200*	0	0	0
133	Ethyl di-*n*-butylmalonate	259/—	K.	180*	30	30	30
215	N,N̂-dicyclohexylimadazolidine	125/0.5	T.W.	50	480	480	510
172	Diphenylcyclopropane	140/12	T.W.	168*	0	0	0
185	Diphenyl ether	259/—	E.K.	189	0	30	30
140	2,2-Dimethyl-5-(1,3,3-trimethylpentyl)-dioxolone-4	115/15	T.W.	138	30	30	30
141	2-Methyl-2-ethyl-5-(1,3,3-trimethylpentyl)-dioxolone-4	107/1.0	T.W.	98	30	30	30

** At the beginning of the test; or else, after 30 min.*

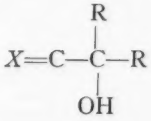
Some of these compounds were tested because they were intermediate in the synthesis of other compounds described in this paper. Many were tested in a random search for other effective arrangements of molecules known to be repellent. The great number of negative results indicates that good repellency is specific to a few functional groups.

The exception to the ineffectiveness of miscellaneous types in Table VII is N,N̂-dicyclohexylimidazolidine, with a repellency time of 480 min. to the first bite. This effectiveness might not have been predicted from its tertiary amino structure or from its high boiling point.

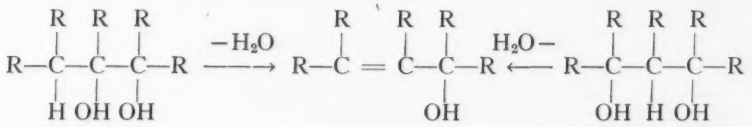
Conclusions

If compounds containing nitrogen are excluded, the important atom from the standpoint of repellency seems to be oxygen. This agrees with the opinion of Bunker and Hirshfelder (1). However, an oxygenated linkage such as hydroxy or carbonyl alone does not induce unusual repellency.

Dimethyl phthalate is an exception to this rule. Enhanced powers of repellency seem to result when an hydroxyl group is once removed from unsaturation, i.e.,



where X may be oxygen or carbon. Glycols (1,2 or 1,3) may be included in this category in so far as their dehydration products conform with this type:—



1,2-Glycol 1,3-Glycol

The odor conferred by this grouping of atoms may not in itself be repellent to insects, but may increase olfactory sensitivity so that the objectionable group is more easily perceived. It is suggested that the phenyl group is repellent. Experiments in progress should serve to confirm or refute these opinions.

It seems probable that the repellency of a substance is related to the effective vapor concentration at which it can be maintained. The maximum repellent activity was invariably exhibited by compounds whose atmospheric boiling points ranged from 230° to 260° C. Presumably such chemicals maintain an effective vapor concentration. The determination of this factor is not easy since it is certainly not wholly related to the boiling point but probably also to the vapor pressure of the substance at the temperature of the animal skin. The association of vapor concentration with repellency may be important, but the study of this physicochemical problem was not undertaken.

The enhancement of repellency by the use of mixtures, which was reported by Jackowski and Pijoan (4), has been confirmed in this study.

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References

1. BUNKER, C. W. O. and HIRSHFELDER, A. D. Mosquito repellents. Am. J. Trop. Med. 5 : 359. 1925.
2. GRANETT, P. Studies of mosquito repellents. I. Test procedure and method of evaluating test data. J. Econ. Entomol. 33 : 563-565. 1940.
3. GRANETT, P. and HAYNES, H. L. Improved methods of rearing *Aedes aegypti* mosquitoes for use in repellent studies. Proc. 31st Annual Meeting New Jersey Mosquito Exterminaters Assoc. 161-168. 1944.
4. JACKOWSKI, L. A. and PIJOAN, M. Two new effective insect repellents, NMRI-201 and NMRI-448. Science, 104 : 266-269. 1946.
5. MORTON, F. A., TRAVIS, B. V., and LINDUSKA, J. P. Part V. Evaluation of materials as mosquito repellents. *In* Results of screening tests with materials evaluated as insecticides, miticides and repellents at the Orlando laboratory, April 1942 to April 1947, pp. 10-11, 22-235. U.S. Dept. Agr., Bur. Entomol. and Plant Quarantine, E-733. 1947.
6. TREMBLEY, H. L. Mosquito culture techniques. Mosquito News, 4 : 103-119. 1944.

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